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Prediction of the metabolisable energy content of forages and mixed diets for horses: validation and comparison of two evaluation systems

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ABSTRACT

The metabolisable energy (ME) content of feeds is a better estimate of their 'true' energy value than their digestible energy (DE) content, because ME takes account of the gross energy of methane (GE_{gas}) and the gross energy of urine (GE_{urine}) losses. The accuracy and precision of the Gesellschaft für Ernährungsphysiologie (GfE) and Institut National de la Recherche Agronomique (INRA) systems for predicting the DE and ME contents of diets for horses were compared using the results of a study comprising 15 mixed diets. The INRA system was more accurate than the GfE system for predicting DE, GE_{urine} and ME: the biases between the predicted and the measured values were -0.26 vs -0.46 MJ/kg DM for DE ($P < 0.05$), -0.03 vs 0.13 MJ/kg DM for GE_{urine} ($P < 0.05$) and -0.09 vs -0.62 MJ/kg DM for ME ($P < 0.05$). The biases for GE_{gas} were not significantly different ($P > 0.05$) between systems. In addition, a study was carried out with 24 forages to compare the ME value of permanent meadow and lucerne hays predicted with the GfE and the INRA systems. The INRA system gave higher prediction values of DE than the GfE system ($P < 0.001$) and lower estimates of GE_{gas} (0.34 vs 0.63 MJ/kg DM for permanent meadow hays and 0.38 vs 0.63 MJ/kg DM for lucerne hays) ($P < 0.001$) and GE_{urine} (0.85 vs 0.93 MJ/kg DM for grassland hays and 1.08 vs 1.37 MJ/kg DM for lucerne hays) ($P < 0.001$). The INRA system thus gave higher estimates of ME (7.57 vs 6.77 MJ/kg DM for permanent meadow hays and 8.80 vs 6.46 MJ/kg DM for lucerne hays, $P < 0.001$) in agreement with the results obtained with mixed diets. The ME values of permanent meadow hays and legume hays should therefore be predicted separately using specific equations as previously established for the DE value.

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Implications

Horses are mainly fed forage-based diets. The energy value of forages is currently evaluated using different feed evaluation systems. The present study shows that the metabolisable energy value of mixed diets predicted with the Institut National de la Recherche Agronomique system is more accurate than that predicted by the Gesellschaft für Ernährungsphysiologie system. Differences between systems for predicting metabolisable energy of hays were greater for lucerne than for permanent meadows. The energy requirements of horses can thus be met more accurately using the Institut National de la Recherche Agronomique evaluation system.

Introduction

The population of horses bred and used for leisure activities; equestrian sports and racing is now large in most industrialised countries. Feedstuffs are chosen for their good palatability to minimise feed

refusals, energy and protein losses and feed residues (methane, ammonia); their rationing is set to meet the nutrient requirements of horses and control feeding costs. Horses are daily fed 50 to 90% forage-based diets according to feeding recommendations (Institut National de la Recherche Agronomique (INRA), 2015). The energy value of forages or concentrates and mixed diets is currently evaluated using various feed evaluation systems. In a previous study (Martin-Rosset et al., 2020), we showed that the digestible energy (DE) value of hays predicted by the Institut National de la Recherche Agronomique (INRA) system was more accurate than that from the Gesellschaft für Ernährungsphysiologie (GfE) and National Research Council systems provided that the botanical family (permanent meadow vs lucerne) and relevant chemical criteria were considered. The main reason for this discrepancy could be the use of different equations to predict the DE content of natural grassland or legume hays in the INRA system. However, no validation study has been carried out with mixed diets although the components of concentrate could influence the performance of the different systems, nor have any comparison studies been performed on different systems for predicting the metabolisable energy (ME). The ME content of feeds is a better estimate of their 'true' energy value than their DE content, because ME takes into account the gross energy of methane

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gas (GE_{gas}) and the gross energy of urine (GE_{urine}) losses (Vermorel and Martin-Rosset, 1997; Institut National de la Recherche Agronomique (INRA), 2015). The ME content of feeds depends on their chemical composition, especially their CP and crude fibre (CF) contents (Fingerling, 1953; Hoffmann et al., 1967; Vermorel and Martin-Rosset, 1997; Kienzle and Zeyner, 2010). We hypothesised that the difference in DE between the two systems might be altered by differences in the predicted methane and urinary energy losses, and in different ways for grassland and legumes, like for DE (Martin-Rosset et al., 2020), and possibly for mixed diets. The aims of the present study were (i) to validate the GfE and INRA systems for predicting the ME value of mixed diets for horses using two independent studies and (ii) to compare the ME contents of two sets of permanent meadow and lucerne hays predicted with the two systems.

Material and methods

Concepts of the Gesellschaft für Ernährungsphysiologie and the Institut National de la Recherche Agronomique systems for predicting the metabolisable energy value of feeds

The predicted ME content of forages is derived from their DE content by removing the methane and urinary energy losses.

Gesellschaft für Ernährungsphysiologie system

GE_{gas} and GE_{urine} were estimated to be 2.0 ± 0.6 kJ/g CF and 8.0 ± 1.3 kJ/g CP, respectively, from a literature review (Kienzle and Zeyner, 2010). The GfE Scientific Committee computed Eq. (1) to predict the DE content of feeds and Eq. (2) to predict their ME content from their chemical composition (CP, ether extract, CF and nitrogen-free extract) (Gesellschaft für Ernährungsphysiologie (GfE), 2014).

$$\text{DE (MJ/kg DM)} = -3.54 + (0.0209 \text{ CP}) + (0.0420 \text{ ether extract}) + (0.0001 \text{ CF}) + (0.0185 \text{ nitrogen-free extract}) \quad (1)$$

$$\text{ME (MJ/kg DM)} = -3.54 + (0.0129 \text{ CP}) + (0.0420 \text{ ether extract}) - (0.0019 \text{ CF}) + (0.0185 \text{ nitrogen-free extract}) \quad (2)$$

Eq. (1) is based on a data file of 170 trials extracted from the literature. The trials were carried out with horses fed close to maintenance, mainly with mixed diets (hays or native or treated straws supplemented with grains or grains plus protein sources or with by-products, and some forage-based diets) or forages (hays, haylage, straws plus minerals and protein supplements to balance the diets and prevent impaired feeding behaviour) (Kienzle et al., 2002). The CF and CP contents were in the ranges 42–347 g/kg DM and 57–287 g/kg DM, respectively. The ether extract content should be lower than 80 g/kg DM (Zeyner, 1995). The DE value of feeds was computed from the contents of their main chemical components, the corresponding digestibility coefficients (the CF digestibility coefficient was set at 0.47) and their GE contents (Zeyner and Kienzle, 2002).

The methane and urinary energy losses were computed from 105 data points from the literature and used to compute the predictive Eq. (2) (Kienzle and Zeyner, 2010; Gesellschaft für Ernährungsphysiologie (GfE), 2014).

Institut National de la Recherche Agronomique system

The DE value of feeds is calculated from GE of organic matter (Eq. (3)) followed by calculating digestibility of organic matter (Eqs. (4) and (5)) which is then converted into digestibility of energy (Eq. (6)) in a stepwise approach and then applied to the GE content (Eq. (7)) (Institut National de la Recherche Agronomique (INRA), 2015).

$$\begin{aligned} \text{Gross energy : GE (kcal/kg organic matter)} \\ = 4531 + 1.735 \text{ CP (g/kg of organic matter)} \\ \pm \Delta \text{ (according to the type of forage)} \end{aligned} \quad (3)$$

$$\begin{aligned} \text{Digestibility of organic matter (\%)} \\ = 87.89 - 0.1180 \text{ CF content for permanent meadows} \end{aligned} \quad (4)$$

$$\begin{aligned} \text{Digestibility of organic matter (\%)} \\ = 90.52 - 0.0995 \text{ CF content for legumes} \end{aligned} \quad (5)$$

$$\begin{aligned} \text{Digestibility of energy (\%)} &= 0.0340 + 0.9477 \\ &\times (\text{digestibility of organic matter; (\%)}) \pm \Delta \end{aligned} \quad (6)$$

(where $\Delta = -1.1$ for forages; $\Delta = 1.1$ for concentrates)

$$\text{DE (kcal/kg DM)} = \text{GE} \times (\text{digestibility of energy; (\%)}) \quad (7)$$

The ME content of feeds is predicted as $\text{ME} = \text{DE} \times (\text{ME/DE})$. For that purpose, a study was, prior to the current work, carried out at INRA with 26 horses: six or eight animals warmblood breeds per treatment fed near maintenance 12 diets (five forage diets and seven mixed diets) to measure diet digestibility, GE_{gas} and GE_{urine} (Vermorel et al., 1997a). The ADF content of the diets ranged from 204 to 413 g/kg DM, the CF content from 186 to 381 g/kg DM, the CP content from 75 to 142 g/kg DM and the water-soluble carbohydrates content from 36 to 258 g/kg DM. Diet digestibility was determined by the total *in vivo* collection of faeces over at least 5 consecutive days after 2 weeks of adaptation to the diet (according to Martin-Rosset et al., 1984). Methane and urinary energy losses were measured in respiration chambers for 4 and 6 consecutive days, respectively, after adaptation to the chambers (79 individual data, Vermorel and Martin-Rosset, 1997). The validity of methane measurements was checked gravimetrically by infusion of methane into the chambers for 6 to 8 h after equilibrium. Urinary energy was determined by bomb calorimetry after freeze-drying in small polythene bags. Seventy-nine determinations were obtained to compute prediction equations of methane and urinary energy losses and then predict ME/DE (%):

$$\begin{aligned} \text{GE}_{\text{gas}}/\text{energy intake (EI) (\%)} \\ = 4.56 - 0.008 \text{ ADF} - 0.002 \text{ Water soluble carbohydrates} \\ R^2 = 0.46, \text{RSD} = 0.41 \end{aligned} \quad (8)$$

$$\begin{aligned} \text{GE}_{\text{urine}}/\text{EI (\%)} &= 6.10 + 0.022 \text{ CP} - 0.011 \text{ ADF} \\ &\quad - 0.006 \text{ Water soluble carbohydrates} \\ R^2 &= 0.48, \text{RSD} = 0.63(9) \end{aligned} \quad (9)$$

$$\begin{aligned} \text{ME/DE (\%)} &= 83.42 - 0.024 \text{ CP} + 0.016 \text{ ADF} \\ &\quad + 0.019 \text{ Water soluble carbohydrates} \\ R^2 &= 0.46, \text{RSD} = 1.36 \end{aligned} \quad (10)$$

However, the ADF content of feeds or diets had not been determined in the studies of Fingerling (1953) and Hoffmann et al. (1967). The ADF content of the permanent meadow hays used was predicted from their CF content using the following equation: $\text{ADF (g/kg DM)} = 0.83 \text{ CF} + 76$ $R^2 = 0.99$, $\text{RSD} = 4.0$ (Institut National de la Recherche Agronomique (INRA), 2015). The ADF content of concentrate feeds was extracted from the values of similar feeds tabulated in the tables of the Institut National de la Recherche Agronomique (INRA) (2015).

Experimental databases

Step 1: diets used for the validation of the Gesellschaft für Ernährungsphysiologie and the Institut National de la Recherche Agronomique systems to predict metabolisable energy

The results obtained from 13 mixed diets studied by Fingerling between 1931 and 1939, (Fingerling, 1953) and two diets studied by Hoffmann et al. (1967) were used for the validation study. The 15 diets consisted of a basal diet composed of $68.6 \pm 9.44\%$ permanent meadow hay (7 different batches) and $31.4 \pm 9.44\%$ concentrate feeds (oats, linseed meal and molasses). The basal diet was supplemented with permanent meadow hay or various usual concentrate feeds. The chemical composition of the mixed diets reported by the authors was the following on the DM basis: CP: 136 ± 13 g/kg, CF: 255 ± 27 g/kg, ether extract: 30 ± 3 g/kg, nitrogen-free extract: 512 ± 41 g/kg. However, the water-soluble carbohydrates content of the constituting feeds were estimated from the values tabulated in the French feeding tables for similar feeds (Institut National de la Recherche Agronomique (INRA), 2015); they averaged 155 ± 49 g/kg. Both studies determined *in vivo* digestibility by a total collection of faeces over 6-day periods after a 10–14-day adaptation period. Urine and methane emissions were measured for 6 and 4 consecutive days, respectively. The DE and ME contents of the 15 mixed diets predicted in the GfE system were calculated from their chemical composition using Eqs. (1) and (2). By contrast, the DE content predicted in the INRA system was calculated using the stepwise method (Eqs. (3) to (7)) and the ME content using Eq. (10).

Step 2: comparison study: comparison of the Gesellschaft für Ernährungsphysiologie and the Institut National de la Recherche Agronomique systems for predicting metabolisable energy of permanent meadow and lucerne hays

Two new batches of 15 permanent meadow hays and nine lucerne hays were used to test the validity of the GfE and INRA systems to predict the DE content of hays. The botanical composition of permanent meadow hays was not determined but according to the grassland types reported by Andueza et al. (2016) in the same area, we can consider that they were mostly comprised of grasses. Lucerne hays were composed of pure lucerne as far as they were second and third cut. Their chemical composition and *in vivo* measured DE contents had been published previously (Martin-Rosset et al., 2020). However, GEgas and GEurine had not been measured; they were predicted with the INRA system using Eqs. (8) and (9). The ME contents of feeds were predicted using Eq. (2) in the GfE system, and in the INRA system from their predicted DE contents (Eqs. (3) to (7)) and then using Eq. (10).

Statistical analysis of results

Sample values of the database composed by Fingerling (1953) and Hoffmann et al. (1967) were first predicted for ED, EM, GEgas and GEurine according to the models proposed by the GfE and INRA systems. The ratios GEgas/EI, GEurine/EI and EM/ED were also calculated from the data of each system. For each determination, the means and SD were first calculated for the measured values and the predicted values obtained by each system. Linear regression models between measured and predicted values from each system were then developed for each determination. The models obtained were assessed by the R^2 statistic and the standard error of prediction (SEP). The SEP was decomposed into the bias and the SEP was corrected by the bias (SEP(C)). Values of bias and SEP(C) obtained for models of each system were compared using the procedure proposed by Fearn (1996). Analyses were performed using the mixed procedure of the Statistical Analysis System statistics package (Statistical Analysis System (SAS), 1998).

Data obtained from the comparison study were tested by ANOVA according to the following model:

$$Y_{ijkl} = \mu + F_i + R(F)_{ij} + S_k + (F \times S)_{ik} + \varepsilon_{ijkl}$$

where Y is the dependent variable, μ is the overall mean, F is the type of forage (1 degree of freedom df), S is the prediction value obtained by each system (1 df), $F \times S$ is the interaction between type of forage and system (1 df), R is the replication nested to the type of forage (22 df), and ε is the experimental error (22 df). Replication was considered as a random effect. Analyses were performed using the mixed procedure of the Statistical Analysis System statistics package (Statistical Analysis System (SAS), 1998). Significance was set at $P < 0.05$.

Results

Step 1: validation of the Gesellschaft für Ernährungsphysiologie and the Institut National de la Recherche Agronomique systems

The mean DE and ME contents of the 15 mixed diets predicted by the GfE and the INRA systems were lower than the *in vivo* measured DE and ME contents, whereas the predicted GEgas and GEurine were lower in the INRA system, but higher in the GfE system (Table 1). The R^2 values of the regression model were above 0.86 for DE and ME but very low for GEgas and GEurine in both systems (Table 2). The SEP values of DE, GEurine and ME were lower in the INRA system than in the GfE system (Table 2). The biases for DE averaged -0.46 MJ/kg DM and -0.26 MJ/kg DM ($P < 0.05$) for the GfE system and the INRA system,

Table 1

Measured values (MV) and values predicted by the Gesellschaft für Ernährungsphysiologie (GfE) and the Institut National de la Recherche Agronomique (INRA) horse feed evaluation systems for energy values and energy losses (mean $\pm$ SD) of thirteen mixed diets studied by Fingerling (1953) between 1931 and 1939 and two diets studied by Hoffmann et al. (1967).

	MV	INRA	GfE
DE (MJ/kg DM)	10.53 ± 0.749	10.24 ± 0.618	10.04 ± 0.604
GEgas (MJ/kg DM)	0.41 ± 0.132	0.36 ± 0.020	0.51 ± 0.058
GEgas (%EI)	2.37 ± 0.410	1.95 ± 0.107	2.76 ± 0.293
GEgas (kJ/g CF)	1.77 ± 0.356	1.43 ± 0.230	2 ± 0.6^1
GEurine (MJ/kg DM)	0.96 ± 0.140	0.93 ± 0.074	1.09 ± 0.112
GEurine (%EI)	5.18 ± 0.760	5.02 ± 0.399	5.90 ± 0.576
GEurine (kJ/g CP)	7.10 ± 1.041	6.83 ± 0.211	8 ± 1.3^1
ME (MJ/kg DM)	9.08 ± 0.721	8.98 ± 0.603	8.46 ± 0.695
ME/DE (%)	86.6 ± 1.75	87.6 ± 1.10	84.3 ± 2.15

DE: digestible energy; GEgas: methane energy; GEurine: urinary energy; ME: metabolisable energy; ME/DE: metabolisable energy expressed as percentage of DE; EI: energy intake; CF: crude fibre.

¹ Values fixed by GfE System.

Table 2

Standard error of prediction (SEP) and coefficient of determination (R^2) values obtained when the Gesellschaft für Ernährungsphysiologie (GfE) and the Institut National de la Recherche Agronomique (INRA) horse feed evaluation systems were applied to the validation set to predict energy values and energy losses.

	INRA		GfE	
	SEP	R^2	SEP	R^2
DE (MJ/kg DM)	0.37	0.86	0.52	0.89
GEgas (MJ/kg DM)	0.09	0.02	0.10	0.009
GEgas %EI	0.50	0.06	0.54	0.001
GEgas (kJ/g CF)	0.37	0.45	0.38	0.01
GEurine (MJ/kg DM)	0.14	0.11	0.19	0.11
GEurine %EI	0.74	0.10	1.05	0.09
GEurine (kJ/g CP)	0.98	0.06	1.33	0.01
ME (MJ/kg DM)	0.25	0.89	0.63	0.96
ME/DE (%)	1.68	0.36	2.98	0.29

DE: digestible energy; GEgas: methane energy; GEurine: urinary energy; ME: metabolisable energy; ME/DE: metabolisable energy expressed as percentage of DE; EI: energy intake; CF: crude fibre.

Table 3

Bias and standard error of prediction corrected for bias (SEP(C)), for energy values and energy losses obtained when the predictions with Gesellschaft für Ernährungsphysiologie (GfE) and the Institut National de la Recherche Agronomique (INRA) horse feed evaluation systems were applied to the validation set.

	Bias			SEP(C)		
	INRA	GfE	P-value	INRA	GfE	P-value
DE (MJ/kg DM)	−0.26	−0.46	<0.05	0.27	0.25	>0.05
GEgas (MJ/kg DM)	−0.08	0.07	>0.05	0.05	0.07	>0.05
GEgas %EI	−0.43	0.38	>0.05	0.27	0.40	>0.05
GEgas (kJ/g CF)	−0.30	0.26	>0.05	0.22	0.29	>0.05
GEurine (MJ/kg DM)	−0.03	0.13	<0.05	0.14	0.15	>0.05
GEurine %EI	−0.18	0.70	<0.05	0.74	0.81	>0.05
GEurine (kJ/g CP)	−0.26	0.91	<0.05	0.97	1.00	>0.05
ME (MJ/kg DM)	−0.09	−0.62	<0.05	0.24	0.14	<0.05
ME/DE (%)	0.99	−2.35	<0.05	1.41	1.91	>0.05

DE: digestible energy; GEgas: methane energy; GEurine: urinary energy; ME: metabolisable energy; ME/DE: metabolisable energy expressed as percentage of DE; EI: energy intake; CF: crude fibre.

respectively (Table 3). For ME they were −0.62 MJ/kg DM; and −0.09 MJ/kg DM ($P < 0.05$) for the GfE system and the INRA system, respectively (Table 3). Bias values were also lower when the INRA system was used to predict GEurine and ME/DE than when the GfE system was used. No statistically significant differences between systems were observed for bias for the predictions of GEgas (Table 3). For SEP (C) no significant differences were observed between systems for all determinations except for ME. For ME the SEP(C) was lower with the GfE system than with the INRA system (Table 3).

Step 2: comparison of the Gesellschaft für Ernährungsphysiologie and the Institut National de la Recherche Agronomique systems

The mean ME content of the 15 permanent meadows predicted in the GfE system was 0.80 ± 0.346 MJ/kg DM (i.e. $10.5 \pm 4.3\%$) lower than that predicted in the INRA system ($P < 0.001$; Table 4). The difference between systems for predicting the mean DE content was -0.39 ± 0.351 MJ/kg DM, ($P < 0.001$) and it was 0.29 MJ/kg DM; ($P < 0.001$) for the differences in the GEgas. However, GEurine predicted contents were 0.85 and 0.93 MJ/kg DM, ($P < 0.001$) for INRA and GfE, respectively. The ME/DE ratio was 5.8 points lower in the GfE than in the INRA system ($P < 0.001$; Table 4).

The mean ME content of the 9 lucerne hays predicted in the GfE system was 2.34 ± 0.531 MJ/kg DM ($-26.5 \pm 5.52\%$) lower than that predicted in the INRA system ($P < 0.001$) (Table 4). The difference between systems for DE content was -1.86 ± 0.508 MJ/kg DM ($P < 0.001$), for GEgas was 0.25 ± 0.093 MJ/kg DM ($P < 0.001$) and for GEurine was 0.29 ± 0.053 MJ/kg DM ($P < 0.001$) (Table 4). The ME/DE (%) was

therefore 9.0 points lower ($P < 0.001$) with the GfE than with the INRA system.

The interaction between the botanical family and the system was highly significant ($P < 0.001$) for all determinations except for GEgas (Table 4). For DE, GEurine, ME and ME/DE the differences between the INRA and GfE system were greater ($P < 0.01$) for lucerne than for the permanent meadows.

Discussion

Validation of the Gesellschaft für Ernährungsphysiologie and the Institut National de la Recherche Agronomique systems

Very few *in vivo* data are available on the ME content of mixed diets fed to gelding horses near the maintenance feeding level. The results obtained by Fingerling (1953) with two horses fed 13 mixed diets usable in practice and those obtained by Hoffmann et al. (1967) with two horses fed two mixed diets were therefore used for the validation study. The results obtained with only one horse, and those obtained with experimental diets composed of a basal diet supplemented by purified compounds (sucrose, extracted cellulose, groundnut oil, etc.) used by Fingerling (1953) were discarded.

The results of the validation study carried out with 15 mixed diets from the literature, showed a lower prediction error of DE with the INRA system than with the GfE system, mainly due to the lower biases. The *in vivo* data were indeed obtained with only two horses per treatment but the results are in agreement with those of a previous validation study on permanent meadow hays and lucerne hays carried out with 5 to 8 horses per treatment (Martin-Rosset et al., 2020). The prediction of the ME content of the mixed diets was also more accurate with the INRA system than with the GfE system, the bias being significantly lower. These differences may result from the sources of data used to predict the GEgas, GEurine and ME values. With the GfE system, the mean values of GEgas (2.0 ± 0.6 kJ/g CF) and GEurine (8.0 ± 1.3 kJ/g CP; Kienzle and Zeyner, 2010; Gesellschaft für Ernährungsphysiologie (GfE), 2014) were computed from the results obtained in four studies with different types of horses fed mixed diets at various feeding levels. The mean GEgas (kJ/g CF) and GEurine (kJ/g CP) averaged 1.59 ± 0.470 and 7.10 ± 1.120 , respectively, in geldings fed near maintenance (Fingerling, 1953; Hoffmann et al., 1967; Vermorel et al., 1997a); 2.50 ± 0.292 and 8.01 ± 1.415 in pregnant mares, 2.72 ± 0.320 and 6.50 ± 0.512 in lactating mares, 4.28 ± 0.918 and 10.29 ± 0.869 in suckling foals and 1.73 ± 0.060 and 9.36 ± 0.278 in weaned foals (Burlacu et al., 1993). The wide variability of the data shows that GEgas/EI and GEurine/EI vary with the physiological status of horses, the chemical composition of the feeds or diets and the feeding level; these factors should be taken into account to predict GEgas and GEurine.

Interestingly, the bias of DE was 1.8 times higher in the GfE system than in the INRA system (0.46 vs 0.26 MJ/kg DM), while the bias of

Table 4

Average values predicted by the Gesellschaft für Ernährungsphysiologie (GfE) and the Institut National de la Recherche Agronomique (INRA) horse feed evaluation systems for 15 permanent meadows and 9 lucerne hays obtained at INRA (Martin-Rosset et al., 2020) and significance levels obtained in the analysis of variance developed for energy values and energy losses.

	PM		Lucerne		SEM (F)	SEM (S)	SEM (F*S)	P-value		
	INRA	GfE	INRA	GfE				F	S	F*S
DE (MJ/kg DM)	8.71	8.32	10.32	8.46	0.30	0.22	0.31	>0.05	<0.001	<0.001
GEgas (MJ/kg DM)	0.34	0.63	0.38	0.63	0.02	0.02	0.03	>0.05	<0.001	>0.05
GEgas %EI	1.87	3.43	2.06	3.40	0.11	0.11	0.15	>0.05	<0.001	>0.05
GEurine (MJ/kg DM)	0.85	0.93	1.08	1.37	0.09	0.06	0.09	<0.05	<0.001	<0.001
GEurine %EI	4.57	5.00	5.83	7.35	0.45	0.32	0.45	<0.01	<0.001	<0.001
ME (MJ/kg DM)	7.57	6.77	8.80	6.46	0.23	0.17	0.24	>0.05	<0.001	<0.001
ME/DE (%)	87.2	81.4	85.3	76.3	0.53	0.46	0.65	<0.001	<0.001	<0.001

PM: permanent meadows; DE: digestible energy; GEgas: methane energy; GEurine: urinary energy; ME: metabolisable energy; ME/DE: metabolisable energy expressed as percentage of DE; EI: energy intake; F: forage; S: system; F*S: interaction forage*system; SEM (F): SEM for comparison between forages mean values; SEM (S): SEM for comparison between systems mean values; SEM (F*S): SEM for comparison between forage*system mean values.

ME was 6.9 times higher (-0.62 vs -0.09 MJ/kg DM). Furthermore, the difference between ME values predicted in the two systems (0.52 MJ/kg DM) was 2.6 times higher than the difference in the predicted DE values (0.20 MJ/kg DM) in the GfE system than in the INRA system. The difference in DE content predicted with the GfE system accounted for 38% of the difference in ME content of mixed diets. The GfE models for predicting DE and ME were developed using different types of horses, whereas the models proposed by the INRA system were built in conditions similar to those used in the validation set (gelding warmblood horses near maintenance). These differences in how the model was developed could explain the bias differences between the systems.

However, the INRA system is less precise than the GfE system for predicting ME, as shown by the higher SEP(C). This might be because the INRA system use the Eq. (10) to predict the percentage of ME/DE which was computed from the results obtained with 43% forage-only diets and 57% mixed diets. Their CF content ranged from 168 to 381 g/kg DM and the CP content from 71 to 142 g/kg DM. However, in the GfE system the prediction equation for ME was computed from a large set of data composed of 15% forage-only diets and 85% of mixed diets including concentrate feeds as far as the CF content ranged from 42 to 347 g/kg DM and CP content between 57 to 287 g/kg DM. Concentrate feeds were probably fed alone as well since some CF contents were as low as 47 g/kg DM (Zeyner and Kienzle, 2002). This range of variation is wider than the INRA range, especially in the area of concentrate feeds, which could result in a better precision of ME prediction for mixed diets. Although SEP(C) for ME obtained with the GfE system was more precise than that obtained with the INRA system, the range of the SEP(C) was much lower than that of the bias.

Step 2: comparison of the Gesellschaft für Ernährungsphysiologie and the Institut National de la Recherche Agronomique systems

The predicted DE value of hays varied with the prediction system and the botanical family. Contrary to the GfE system in which the DE content of all hays were predicted by the same model (Kienzle and Zeyner, 2010; Gesellschaft für Ernährungsphysiologie (GfE), 2014), in the INRA system the DE contents of permanent meadow hays and legumes hays are predicted using different prediction equations of organic matter digestibility obtained with 28 and 25 forages, respectively, and 6 horses fed near maintenance per treatment (Martin-Rosset et al., 1984; Institut National de la Recherche Agronomique (INRA), 2015). Taking into account the differences in NDF and ADF contents between grasses and legumes, the use of a model per family, for predicting the DE content in the INRA system could explain their more precise results. The differences in ME content of feeds predicted in the two systems were greater than the differences in predicted DE content because of higher GE_{gas} and GE_{urine} losses predicted in the GfE system. In the present study, GE_{gas} was predicted in the INRA system using Eq. (8). The latter was established in a previous study from 79 *in vivo* measurements of 4-day methane emissions with six to eight horses per treatment fed 12 forage or mixed diets, after calibration by methane infusion into the respiration chambers: the recovery was $100.0 \pm 0.9\%$ (Vermorel et al., 1995). By contrast, in the GfE system the higher values (GE_{gas} = 2 kJ/g CF, and GE_{urine} = 8 kJ/g CP) may result from the various data used, as discussed above. The prediction Eq. (2) of ME derived from the prediction Eq. (1) of DE using the above correction factors for CF and CP.

The effect of the botanical family (permanent meadow hays vs lucerne hays) and the interaction with the system (INRA vs GfE) on the prediction of DE and ME were significant. The differences in DE content account for 49 and 80% for permanent meadow and lucerne hays, respectively, of the differences in ME content.

Urinary nitrogen and energy losses increase with CP intake (Slade et al., 1970; Reitnour and Treece, 1971; Hintz and Schryver, 1972; Kienzle and Zeyner, 2010). Ragnarsson and Lindberg (2008) pointed

out that those losses are higher at the first early cut than at the late cut in Icelandic horses fed timothy haylages probably due to the variation of the non-protein nitrogen (NPN)/protein nitrogen (PN) ratio in the haylage. However, the NPN/PN ratio depends also on the preservation method (silage, haylage or hay) and on the conditions of harvest (Demarquilly et al., 1981). Amino-acid composition of CP might be involved in the variation of urinary nitrogen losses at least with mixed diets (Slade et al., 1970; Reitnour and Treece, 1971; Hintz and Schryver, 1972) but probably not with forages. Contrary to concentrates, the amino-acid composition of protein of forages does not vary very much according to the botanic family, species and age of the plant (Demarquilly et al., 1981). The difference in essential amino acids composition of lucerne versus grasses (cocksfoot and ryegrass) forages averaged -1.27 ± 4.10 g/16 g of N (Demarquilly et al., 1981). This is because 50 to 60% of the total protein content of the forages are represented by a single protein, the ribulose 1–5-diphosphate carboxylase, for example, rubisco (Aufrère et al., 1994). According to these authors, in ruminants, the differences in the ruminal degradation of proteins of forages are related to the variation of the protein structure. The differences in GE_{urine} between the two systems are higher for lucerne hays than for permanent meadow hays, probably because of their higher CP content (171 ± 18.8 g/kg DM vs 116 ± 51.7 g/kg DM). The GfE system predicts GE_{urine} only from the CP content of the diet but does not take into account the effect of some specific end products of the degradation of the cell wall components on urinary energy supplied by energy-rich detoxification compounds such as hippuric acid. According to Hipp et al. (2017), there is a relationship between GE_{urine} and urinary excretion of nitrogen-containing components. The content of phenolic metabolites, namely ferulic acid and *p*-coumaric acid precursors of hippuric acid, are 2 and 4 times higher, respectively, in grasses than in legumes (Hartley and Jones, 1977; Jung et al., 1983). Their content increases by 30% on average with age in grasses but not in legumes, whereas the CP content decreases (Jung et al., 1983). The uptake of CP and phenolic compounds, the absorption and the excretion of metabolites of CP (urea) and phenolic compounds (hippuric acid) of grasses and legumes are widely different. Furthermore, the GE content of hippuric acid, per gram nitrogen is 13.3 times higher than that of urea (Nehring, 1972). Consequently, the mean urinary energy loss of 8.0 kJ/g CP used in the GfE system seems too high for legume hays, and the coefficient assigned to CP in the prediction equation for ME in the GfE system may be overestimated.

From the results of the validation study (Table 3) and those of the comparison study (Table 4), it seems that the ME content of lucerne hays is underestimated in the GfE system because of the lower predicted DE value (Martin-Rosset et al., 2020) and partly because of their higher urinary energy losses related to the higher CP content. In other respects, GE_{gas} and GE_{urine} expressed as a percent of energy intake depends on the type of equines (warmblood horses or ponies) (Vermorel et al., 1997b; Gesellschaft für Ernährungsphysiologie (GfE), 2014), diet composition (Vermorel et al., 1997a; Ragnarsson and Lindberg, 2008; Kienzle et al., 2009; Ragnarsson and Lindberg, 2010), feeding level (Hoffmann et al., 1967; Vermorel et al., 1997a) and on the physiological status of horses: maintenance, growth, pregnancy or lactation, and nitrogen balance (Burlacu et al., 1993). We, therefore, used only data from adult geldings of warmblood breeds fed near maintenance to measure DE and then compute the INRA prediction equation for ME/DE and subsequently the ME value of forages (Vermorel et al., 1997a).

Overall, the differences between the ME values predicted by the GfE system and the INRA system varied with the type of feeds or diets: forages or mixed diets. They result from accumulative discrepancies in DE, GE_{gas} and GE_{urine}, but mainly from discrepancies in predicted DE. The impact of the difference in predicted ME between the two systems on the differences in predicted ME was higher with forage-only diets (65% on average) than with mixed diets (38%) and much higher for lucerne hays (80%) than for permanent meadows hays (49%).

Conclusion

The validation study carried out with 15 mixed diets from the literature shows that the INRA system predicts their DE and ME contents more accurately than the GfE system. In addition, the comparison study carried out with 15 permanent meadow hays and nine lucerne hays shows that the GfE system underestimates their DE content and predicts higher GE_{gas}, GE_{urine} and consequently lower ME contents of forages, especially those of lucerne hays, than the INRA system. Hence, the ME content of permanent meadow hays and legume hays should be predicted using different specific equations. Different equations established with data obtained with adult gelding horses in similar physiological status, preferably fed near maintenance, should probably be used for predicting the energy value of hays and mixed forages.

Ethics approval

Not applicable.

Data and model availability statement

None of the data was deposited in an official repository.

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Declaration of interest

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